Published in final edited form as:

Ann Biomed Eng. 2019 June ; 47(6): 1435–1445. doi:10.1007/s10439-019-02242-z.

A Three-Dimensional Model of Human Lung Airway Tree to Study Therapeutics Delivery in the Lungs

Antonio Copploe¹, Morteza Vatani², Jae-Won Choi², and Hossein Tavana^{1,*}

¹Department of Biomedical Engineering, The University of Akron, Akron, OH 44325

²Department of Mechanical Engineering, The University of Akron, Akron, OH 44325

Abstract

Surfactant instillation into the lungs is used to treat several respiratory disorders such as neonatal respiratory distress syndrome (NRDS). The success of the treatments significantly depends on the uniformity of distribution of the instilled surfactant in airways. This is challenging to directly evaluate due to the inaccessibility of lung airways and great difficulty with imaging them. To tackle this problem, we developed a 3D physical model of human lung airway tree. Using a defined set of principles, we first generated computational models of eight generations of neonates' tracheobronchial tree comprising the conducting zone airways. Similar to native lungs, these models contained continuously-branching airways that rotated in the 3D space and reduced in size with increase in the generation number. Then, we used additive manufacturing to generate physical airway tree models that precisely replicated the computational designs. We demonstrated the utility of the physical models to study surfactant delivery in the lungs and showed the effect of orientation of the airway tree in the gravitational field on the distribution of instilled surfactant between the left and right lungs and within each lung. Our 3D lung airway tree model offers a novel tool for quantitative studies of therapeutics delivery.

Keywords

3D lung airway tree; computational design; additive manufacturing; physical models; surfactant delivery

Introduction

The lung contains a series of continuously-branching airways that reduce in size but become more numerous as they penetrate deeper in the lung.¹ The trachea divides into the left and right bronchi, each of which divides into lobar bronchi where the lobes of the lung emerge. This division continues down to terminal bronchioles followed by respiratory bronchioles and alveoli where gas exchange takes place.¹ Type II alveolar epithelial cells synthesize and secrete pulmonary surfactant that adsorbs into the air-liquid interface in the alveolar space

*Corresponding Author: Hossein Tavana, Ph.D., P.Eng., Department of Biomedical Engineering, The University of Akron, 260 S. Forge St., Akron, OH 44325, Tel: (330) 972-6031, Fax: (330) 374-8834, tavana@uakron.edu.

Conflict of Interest

The authors do not have any conflict of interest to declare.

and forms a monomolecular layer.^{2, 3} Lung surfactant reduces the air-liquid surface tension to prevent the microscale respiratory units from collapsing.⁴⁻⁷ Insufficient surfactant production in the lungs of preterm babies or its inactivation in adult lungs can severely impact respiration, necessitating interventions such as surfactant replacement therapy (SRT) to restore normal breathing.⁸

Lung airways are used as a route of therapeutics delivery to treat certain respiratory conditions such as respiratory distress syndrome (RDS). Delivery of surfactant to alveoli is critical to restore normal respiration.⁹ A surfactant solution is administered into the trachea to form a liquid plug (bolus). Mechanical ventilation is used to push the plug downstream.^{10, 11} The plug splits at some ratio between the left and right primary bronchi. This ratio determines how much surfactant enters into the left and right lungs. The resulting daughter plugs propagate, deposit a trailing film from their rear menisci, and split at each bifurcation into smaller plugs that eventually become very thin and rupture.¹² The coating film drains toward alveoli.⁶ When the film is sufficiently thin, the spreading becomes a kinetic shock wave known as GBG shock.^{13, 14}

The treatments success in large part depends on the distribution of the administered materials in the lungs and whether they reach the intended target zones. In practice, it is extremely difficult to directly evaluate distribution of the administered therapeutics. The treatments effectiveness is often evaluated indirectly through lung function tests and blood oxygenation. Several rounds of treatment may also be used to achieve a desired outcome, although this may lead to the formation of blocking liquid boli in airways and inhomogeneous distribution of the instilled surfactant.¹⁵ Lack of 3D tracheobronchial tree models has been a major obstacle to mechanistically study and optimize therapeutics delivery in the lungs.

Building upon previous mathematical and computational analysis of liquid plug dynamics in airways,^{16, 17} a major study was recently conducted using mathematical modeling of SRT in symmetric, 3D models of the lung airway tree.¹⁸ Surfactant distribution was computed from fluid mechanical principles to determine splitting of surfactant plugs at airway bifurcations and deposition on the walls. Experimental modeling of SRT has been far less advanced, and studies have primarily focused on propagation of a liquid plug in a straight tube,^{19, 20} an air finger in a straight liquid-filled tube,^{21, 22} splitting of a liquid plug at a bifurcation,^{17, 23} or splitting of an air finger in a liquid-filled branching geometry.²⁴ These bench-top models used microfluidics technology or assembled tubing or plates that did not resemble the shape, length scale, 3D rotation, and wettability of native airways.²⁵ Rat lungs were also used to study delivery of surfactants mixed with a radiopaque tracer for imaging.¹² However, differences between human and rat lungs make it difficult to relate the results to SRT in humans.

To address the need for 3D lung airway models, we developed seamless physical models that represented single bifurcating airways of three successive generations starting from trachea, i.e., $z=0-1$, $z=1-2$, and $z=2-3$.²³ We showed the counteracting roles of gravity and inertia on the splitting of liquid plugs at a bifurcation and increasing effects of surface and viscous forces by reduction in airways size. Building upon this work, here we present a

bioengineering approach to develop the first experimental 3D model of human lung airway tree and its utility to investigate air pressure-driven surfactant delivery and distribution in airways. Our approach offers a new tool to study multi-phase flow and therapeutics delivery in multi-generation models of lung airways.

Materials and Methods

Working Fluid

A clinical surfactant solution, Infasurf (ONY, Inc.), was used at a phospholipids concentration of 35 mg/ml. Dynamic viscosity of the Infasurf solution was measured using a rheometer (Figure S1). A flow rate of 18 ml min⁻¹ was used to propagate Infasurf plugs at a defined Capillary number (Ca). This resulted in a shear rate of 23.95 s⁻¹, translating into a viscosity of 0.24 g cm⁻¹ s⁻¹. The surface tension and density of the Infasurf solution were measured using ADSA as $\gamma=25$ dynes cm⁻¹ and a density meter (DA-100M) as $\rho=0.980$ g cm⁻³, respectively.²⁶

Experimental Setup and Liquid Plug Generation

The airway tree models were fixed on a plexiglass platform with an accelerometer to precisely adjust the gravitational orientation of airway models using a roll angle (α) and a pitch angle (ϕ) (Figure S2).²³ The roll angle determined relative gravitational orientation of daughter tubes in a bifurcating airway unit. When $\alpha>0^\circ$, one daughter tube was gravitationally favored. The pitch angle specified the component of gravity acting along axial direction of the parent tube of a bifurcating airway. When $\phi>0^\circ$, gravity acted along the direction of motion of the plug in the parent tube. Experiments were done at different combinations of α and ϕ measured with respect to the plane of $z=0-1$ generation. Each airway model was exposed to oxygen plasma (Harrick Plasma) for 1 min to render the airways hydrophilic.²³ Next, 75 μ l of the Infasurf solution was injected into the tracheal tube to form a liquid plug. Silicon tubing (Tygon) was connected to the tracheal tube from one end and to a plastic syringe (NormJect) mounted on a positive displacement syringe pump (Chemyx Inc.) from the other end. Pressurized air was used to propagate Infasurf plugs within the airway tree.

Imaging, Image Analysis, and Statistics

Each experiment was recorded as a movie at a rate of 25 fps using an SLR camera (Nikon D3100) equipped with a macro lens (Tamron 272EN II, focusing distance 90 mm). Videos were converted to individual frames to determine plug length in airways. A split ratio was defined as the ratio of lengths or volumes of the two daughter plugs in the upper and lower daughter tubes. Experiments at each orientation had at least ten replicates. To evaluate the effect of orientation of airways on liquid plug splitting, statistical tests were done using one-way analysis of variance (ANOVA) and a Fisher LSD post hoc test ($p<0.05$).

Quantifying the Split Ratio Using Volume Ratio of Daughter Plugs

A) $z=0-1$ bifurcation—For all the measurements taken, subscripts “A” and “B” refer to the left and right lungs, respectively. Additionally, in each bifurcation, subscript “1” indicates the left tube, whereas subscript “2” refers to the right tube. With an orientation of

$\alpha=30^\circ$ and $\varphi=0^\circ$ for the airway tree, the measurements for the $z=0-1$ unit are shown Figure 1a. First, we measured L_{A1} from the beginning of $z=1$ tube where the parent plug split at the $z=0-1$ bifurcation and extended down to the end of the $z=1$ tube where the next bifurcation emerged. Next, we approximated the $z=1-2$ bifurcation zone as an isosceles triangle with the base being equal to the diameter of the $z=1$ tube and calculated L_A . Using these lengths, we calculated the volume of the surfactant solution for a cylindrical column as $V_{A1} = (L_{A1} + L_A/2) * (\pi * R_{A1}^2)$, where R_{A1} represents the radius of the $z=1$ tube denoted by the subscript $A1$. Next, we found the lengths of the two plugs, L_{A11} and L_{A12} , that partially occupied the $z=2$ tubes and calculated the corresponding volume of the surfactant solution as $V_{A11} + V_{A12} = (L_{A11} + L_{A12}) * (\pi * R_{A11}^2)$. The total volume of the plug A, V_A , resulted as $V_A = V_{A11} + V_{A12} + V_{A1}$. Then we estimated the volume of the plug B, V_B , using its length as $V_B = L_B * (\pi * R_B^2)$ and determined the split ratio for the $z=0-1$ bifurcation as $R = V_B/V_A$.

B) $z=1-2$ bifurcations—When the volume of the surfactant solution in the $z=2$ airway tube was larger than the volume of the airway, the surfactant solution also occupied the airway tubes of the subsequent generation $z=3$ in the far-left $z=2-3$ bifurcation. Rotation of the $z=3$ airways 90° with respect to their parent $z=2$ airways made it difficult to record the plugs with our single-camera imaging system. To overcome this limitation, we first measured the length L_{A12} of the plug in the right daughter tube of $z=2$ (Figure 1b) and converted it to a volume, $V_{A12} = L_{A12} * (\pi * R_{A12}^2)$. We also used the total volume of the plug in the left daughter tubes of $z=2$, V_A , which was calculated above. Then, we determined the surfactant solution volume as $V_{A11} = V_A - V_{A12}$, resulting in the split ratio $R = V_{A12}/V_{A11}$ for the $z=1-2$ bifurcation in the left lung.

We also calculated the split ratio for the $z=1-2$ bifurcation of the right lung. Figure 1c shows the plug splitting at this bifurcation. Because the right lung airways are oriented away from gravity in this particular orientation, plugs in these airway tubes were smaller than those in the left lung and did not flow into the subsequent generation airways. This simplified finding the split ratio by measuring the lengths of both plugs, L_{B21} and L_{B22} , and converting them to volumes. This gave the split ratio for the $z=1-2$ bifurcation in the right lung as $R = V_{B22}/V_{B21}$.

We note that if the plugs flowed into the $z=3$ generation airways, the above method could help determine the split ratio. We emphasize that this method allowed us to estimate plug split ratio at $z=2-3$ bifurcations in our proof-of-concept experiment conducted when $\alpha=30^\circ$ and $\varphi=0^\circ$. If the surfactant solution occupied all the $z=3$ airways of the left lung under a different orientation, the above approach could not be used and a more sophisticated imaging would be needed for precise quantitative analysis of this process.

Results

Design Principles of Airway Models

Our design of the geometry of symmetric lung airway tree used five main parameters:

- Diameter (d): The average diameter of parent and daughter airways across the first ten generations of airways follows $d_z = d_0 \times 2^{-z/3}$, where d_0 is the tracheal diameter and z is the generation number.²⁷
- Length (l): The length of each airway is approximately three times its diameter, i.e., $l_z \cong 3d_z$, except for the trachea that follows $l_0 \cong 5d_0$.²⁷
- Tapering: Airways of each generation continuously taper, allowing a smooth transition from one generation of airways to the next one.²⁸
- 3D rotation: Starting at $z=3$, airways rotate $\sim 90^\circ$ in the 3D space with respect to the plane of their parent airway.²⁹ Anatomically, this rotation allows more airways to fit in the chest cavity.
- Bifurcation angle (θ): Bifurcation angle between each two daughter airways is approximately $\theta \cong 80^\circ$.³⁰

We integrated these criteria to computationally design human lung airway tree models in Solidworks software.

Computational Design of a Single Bifurcating Airway

We modeled the trachea and conducting zone airways as rigid tubes because they are cartilaginous and no-deformable during breathing.¹ We created a model of the trachea ($z=0$) by drawing a circle of 3.5 mm diameter, which approximates the tracheal diameter of infants, extending the circle to create a 17.5 mm-long tube to average the length of infants' trachea, and tapering it with a 13.5% decrease in diameter from its opening to its end to enable a smooth transition to the first bifurcation ($z=1$) (Figure 2a). To transition from the $z=0$ to $z=1$ airways, we generated a bifurcation zone as follows.

First, we formed a pair of construction lines, AB and AB', at the bottom of the tracheal tube ($z=0$) and at an angle of $\theta = 80^\circ$ (Figure 2b). Next, we formed a second pair of construction lines from points D and D' perpendicular to AB and AB'. The length of these lines was set as the outer radius of the $z=1$ airways. The lines met at point C, resulting in CD and CD' that defined the opening positions of the daughter tubes (Figure 2b). We formed a plane on each of the lines CD and CD', perpendicular to one of the construction lines AB and AB', respectively, to create the daughter tubes (Figure 2c). We note that the bifurcation zone ends at points D and D'. Therefore, we dimensioned the construction lines AB and AB' with the length of the daughter airways only downstream of D and D'. Then, we formed a circle of known diameter on each of the planes. Each circle passed through the splitting point of the daughter tubes where the bifurcation occurs. Next, we used a 3D feature module in Solidworks known as "lofting" to form a tube of defined thickness between one of the circles and the bottom opening of the tracheal tube (Figure 2d), and repeated it once more to create a second tube and complete the bifurcation zone (Figure 2e). Elongating each daughter tube to accommodate the $l_z = 3d_z$ and the tapering design rules completed the bifurcating airway unit that geometrically replicated infants' trachea to primary bronchi ($z=0-1$) (Figure 2f-g). At the $z=1$ generation, each daughter tube was 2.78 mm and 2.40 mm in diameter at its opening and end, respectively, and 8.33 mm long. We selected a wall thickness of 500 μm to consider the fabrication feasibility. Constructing single bifurcating

airways of other generations follows the steps above, except that the length of each parent tube will be three times its diameter.

Computational Design of a 3D Symmetric Airway Tree Model

We used the bifurcating airway unit as a building block to generate an eight-generation symmetric airway tree model consisting of (2^8-1) tubes. The rationale was to replicate the conducting zone of preterm infants containing generations $z=0-7$ for experimental studies of surfactant distribution in the lungs. Due to the symmetry of the airway tree, we created the model as an “assembly” in Solidworks using a multi-step process. An “assembly” was made by putting together several parts as described below.

A) Start with the $z=0-1$ unit containing only one of the daughter tubes (Figure 3a). The second daughter tube will be added during the assembly process.

B) Follow the protocol for creating a single bifurcating airway that was described above and extend the design from the constructed daughter tube of the $z=0-1$ unit using dimensions of the $z=2$ airway generation (Figure 3b). Again, create only one daughter tube at the bifurcation.

C) Once again, follow the protocol for creating a single bifurcating airway and extend the design from the daughter tube of the $z=2$ generation using dimensions of the $z=3$ airway generation (Figure 3c). Also rotate the bifurcating unit 90° with respect to the plane of its preceding generation. This replicates the rotation of the $z=3$ airways in the 3D space as in native lungs.

D) Repeat step C with dimensions of the $z=4$ airway generation (Figure 3d).

E) Repeat step D, using the appropriate dimensions for the corresponding generations, until the final generation is constructed. To expedite building the airway tree model, create both daughter tubes of the $z=7$ generation (Figure 3e). Save the resulting construct that contains $z=0-7$ generations as a part. This will be used in the assembly process below.

F) Remove the $z=0$ airway and the bifurcation zone that connects it to the $z=1$ airways, leaving generations $z=1-7$. Save this new resulting structure as a part (shown with an arrow in Figure 3f). G) Remove the $z=1$ airway and the bifurcation zone that connects it to the $z=2$ airway. Save the remaining generations $z=2-7$ as a part (shown with an arrow in Figure 3g).

H) Remove the $z=2$ airway and the bifurcation zone that connects it to the $z=3$ airways. Save the remaining generations $z=3-7$ as a part (shown with an arrow in Figure 3h).

I) Continue to reduce the model as above, removing one generation at a time until only the $z=6-7$ generation airways remain and save it as a part (shown with an arrow in Figure 3i). At each step, ensure to only save the remaining structure as a new part, excluding the piece removed.

J) Finally, assemble the model. Start with the part created in step E (Figure 3e) and connect to it the part from step F (Figure 3f) at generation $z=1$ using the “mates” feature in the

assembly section of Solidworks (Figure 4a). Next, use a pair of parts from step G (Figure 3g) and connect them to the z=2 generation of the assembled part from Figure 4a. This will result in the part shown in Figure 4b. Then, use four parts from step H (Figure 3h) and connect them to the z=3 generation of the assembled part from Figure 4b, resulting in the assembled part in Figure 4c. Continue this assembly process until airways of all generations are placed in their respective positions. This process results in a seamless, 3D airway tree model of infants containing eight airway generations of the conducting zone (Figure 4d).

Fabrication of Airway Models

We used the resulting designs to fabricate physical models using a Viper si2 stereolithography system (3D Systems Inc) as vat photopolymerization. Horizontal scans of an ultraviolet (UV) laser beam followed a path defined by the computational model to crosslink a photopolymer (Somos® Watershed SC 11122) layer by layer in a vat.³¹ Figure 5 shows the fabricated models of a single tapering tube, a bifurcating airway, and the eight-generation airway tree model. Importantly, the models are semi-transparent to visualize the interior of the airways. The entire airway tree is a seamless design and fabricated as a single unit. The fabrication method generates very reproducible models. The roughness of interior surface of these models is on the scale of 1–10 μm ,^{32, 33} similar to the roughness of the epithelial cell layer in native airways.^{34, 35} To ensure that the interior surface of airways resemble hydrophilic native airways, we exposed the models to oxygen plasma to render them hydrophilic.²³

Distribution of Instilled Surfactant in Engineered Lung Airways

Surfactant delivery into lungs is a major therapeutic approach to treat several respiratory disorders and improve lung mechanics and respiration. However, incomplete understanding of distribution of instilled surfactant hampers developing delivery strategies to enhance treatments outcome.^{6, 36} Our human lung airway tree model is the first physical model that facilitates imaging the flow of surfactant solution in airways and quantifying its distribution. Thus, we conducted a proof-of-concept study to establish the feasibility of quantitative studies of surfactant delivery in the lung. Due to our limited imaging capability, we used a model with z=0–5 generations. In these experiments, surfactant plugs were propagated using air pressure from a positive displacement syringe pump connected to tracheal tube of the airway models.

A. Split Ratio at z=0–1 and z=1–2 Bifurcations—We formed an Infasurf plug in the z=0 airway (Figure 6a) and propagated it using airflow. Figure 6b shows a propagating plug immediately after splitting at the z=0–1 bifurcation under a horizontal orientation ($\alpha=0^\circ$ and $\phi=0^\circ$). As expected, the plug split evenly between the two daughter airways. Figure 6c shows that at the z=0–1 bifurcation under $\alpha=30^\circ$ and $\phi=0^\circ$, significantly more surfactant solution flowed into the left lung airways that were gravitationally favored. This result is consistent with our previous work using models of single bifurcating airways and a bench-top model of branching airway.^{17, 23} The corresponding videos of splitting of Infasurf plugs are shown in Supplementary Movies.

Starting from the generation $z=3$, airways rotate 90° with respect to their parent airway. This introduces major difficulties with imaging of splitting plugs at bifurcations. For example, Figure 6d–e shows splitting of an Infasurf plug in the $z=1$ – 2 bifurcation of the left lung under $\alpha=30^\circ$ and $\phi=0^\circ$. The plug split unequally between the daughter tubes of the $z=2$ generation and more surfactant solution flowed into the daughter tube on the left. Because the volume of the surfactant solution entering the $z=2$ tube was larger than the volume of the airway, the surfactant solution further occupied the airway tubes of the subsequent generation $z=3$ in the far-left $z=2$ – 3 bifurcation. To overcome the difficulty of capturing plugs in these airways with our single-camera imaging system, we used an approximate approach based on volume of plugs as described above in the Materials and Methods section.

B. Surfactant Distribution in Airways

B.1 Effect of roll angle, $z=0$ – 1 bifurcation: We positioned the airway tree models at three different orientations of $\alpha=30^\circ$, 60° , or 90° , and $\phi=0^\circ$. The rationale for this range of roll angle was based on SRT where babies may be oriented anywhere from lying on their back to completely on one side. We propagated the Infasurf plug in the tracheal tube using airflow, resulting in $Ca=0.030$ at $z=0$ and $Ca=0.024$ at $z=1$ for the plug flow. This was consistent with Ca on a scale of 10^{-2} – 10^{-1} in the trachea during SRT.²³

Increasing the roll angle from $\alpha=0^\circ$ to $\alpha=30^\circ$ caused an asymmetric plug splitting at the $z=0$ – 1 bifurcation and significant drainage of the plug solution into the lower daughter tube ($p<0.05$). The resulting split ratio was $R=0.31\pm0.11$ (Figure 7). Further increase in α to 60° and 90° also significantly reduced the split ratio to 0.08 ± 0.04 and 0.07 ± 0.03 ($p<0.05$), respectively (Figure 7). Thus, rolling the airway tree away from a horizontal position ($\alpha=\phi=0^\circ$) had a major effect on surfactant distribution between the left and right lungs. We expect that a significant increase in Ca will somewhat counteract this gravity-driven drainage into the lower daughter tube of the $z=1$ generation. For example, previous work with single bifurcating airway models at $\alpha=30^\circ$ showed that increasing Ca by an order of magnitude increased the split ratio by 31%, but the effect of gravity was still dominant.²³

B.2 Effect of roll angle, $z=1$ – 2 bifurcation: Plug splitting at the $z=1$ – 2 bifurcation was quite different between the left and right lungs. For the left lung at $Ca=0.024$, increasing α from 0° to 30° caused more than 70% of the plug solution to drain into the lower left daughter tube and gave a split ratio of 0.40 ± 0.07 (Figure 7). Increasing α to 60° and 90° further decreased R to 0.24 ± 0.05 and 0.18 ± 0.06 , respectively. The split ratios at these roll angles were statistically different ($p<0.05$). Therefore, at these orientations, 70–85% of the surfactant solution present in the $z=1$ parent tube drained into the lower left daughter tube of $z=1$ – 2 . This drainage was 75–95% for the lower daughter tube of $z=0$ – 1 . The small difference is likely due to the orientation of the daughter tubes in these generations. That is, in the left lung, the upper daughter tube of the $z=1$ – 2 bifurcation is almost horizontal, whereas the upper daughter tube in the $z=0$ – 1 bifurcation is pointed up and away from the horizontal plane. This slightly reduced the asymmetry of splitting in the $z=1$ – 2 bifurcation of the left lung. In addition, the smaller diameter of airway tubes in $z=1$ – 2 reduced the effect of gravitational draining of the surfactant solution. The Froude number, $Fr = U/\sqrt{gr}$, which

denotes relative effects of inertia and gravity, increased by 1.8 folds from 0.15 at $z=1$ to 0.27 at $z=2$, indicating diminishing effect of gravity with reduced airway diameter.

In the $z=1-2$ bifurcation of the right lung at $Ca=0.024$, increasing α from 0° to 30° produced a minor effect on plug splitting and resulted in a split ratio of 0.95 ± 0.11 ($p>0.05$) (Figure 7). Further increase in α to 60° and 90° reduced the split ratio to 0.77 ± 0.08 and 0.83 ± 0.05 , respectively. Changes in the split ratio from $\alpha=30^\circ$ to $\alpha=60^\circ-90^\circ$ were statistically significant ($p<0.05$) but not within the latter range of α . This result suggests that regardless of the roll angle, the surfactant plug splits almost evenly between the two daughter tubes at $z=2$. We note that by increasing the roll angle, the $z=1$ tube of the right lung moved farther away from a horizontal plane. This resulted in less than ~30% of the initial surfactant solution to enter into the right lung (Figure 6). Our experiments showed that due to the small volume of the plugs in the right lung, they often ruptured before reaching the end of the $z=5$ airways. Therefore, orientations of $\alpha > 30^\circ$ mainly led to surfactant delivery to the left lung.

B.3 Effect of pitch angle, $z=0-1$ bifurcation: We fixed each airway tree model at $\alpha=0^\circ$ or $\alpha=30^\circ$ and then adjusted ϕ to 15° . At the $z=0-1$ bifurcation and with $\alpha=0^\circ$ and $Ca=0.030$, increasing the pitch angle to $\phi=15^\circ$ did not have a significant effect on plug splitting, i.e., $R=1.02\pm0.06$ (Figure 8). However, at $\alpha=30^\circ$ and $\phi=15^\circ$, the splitting became highly asymmetrical and a split ratio of $R=0.11\pm0.06$ resulted. This was a significant reduction of 0.20 in the split ratio at the same roll angle, but without pitching ($p<0.05$) (cf. Figure 8 and 7). This decrease in the split ratio is because pitching the airway tree after rolling it makes the lower daughter tube of $z=0-1$ more gravitationally favored, and a larger volume of the surfactant solution drains into it. Consistent with our previous study,²³ this result also suggests that changing the airway tree orientation using the roll angle has a greater effect on surfactant distribution between daughter tubes emerging from the $z=0-1$ bifurcation than that using the pitch angle.

B.4 Effect of pitch angle, $z=1-2$ bifurcation: For the $z=1-2$ bifurcation of the left lung, introducing a pitch angle had a greater effect on the split ratio than in $z=0-1$. With $Ca=0.024$ and at $\alpha=0^\circ$ and $\phi=15^\circ$, the split ratio significantly increased from unity to $R=1.64\pm0.13$ ($p<0.05$) (Figure 8). This was because the daughter tube on the right-hand side of the bifurcation zone of $z=1-2$ became more gravitationally favored. Increasing α to 30° while keeping $\phi=15^\circ$ opposed the effect and made the daughter tube to the left of the $z=1-2$ bifurcation zone more gravitationally favored, resulting in a significant decrease in the split ratio to $R=0.57\pm0.23$ ($p<0.05$). This was still 0.17 units larger than that without pitching the model (cf. Figures 8 and 7). Therefore, unlike at the $z=0-1$ bifurcation, orienting the airway tree through a pitch angle had a major effect on the distribution of surfactant between airways of the left lung. The significance of the pitch angle effect on plug splitting is shown in the statistical analysis of Table S1. In the $z=1-2$ bifurcation of the right lung, increasing ϕ to 15° and keeping α at 0° made a minor change in the split ratio to $R=1.04\pm0.03$ compared to that at $\alpha=\phi=0^\circ$ (Figure 8). Introducing a roll angle of $\alpha=30^\circ$ reduced the split ratio to 0.82 ± 0.11 , i.e., only 0.13 units smaller than that without pitching the airway tree (cf. Figures 8 and 7).

Discussion

We presented a new approach to develop 3D physical models of lung airway tree. First, we computationally designed a network of successively-branching airway tubes using morphometric data of human lungs. The resulting computational model resembled geometry of native airways in terms of size (diameter, length, and their ratio), rotation of airways of each generation in the 3D space with respect to their parent airways, and a smooth transition of each parent airway to daughter airways through a bifurcation zone. Next, we developed physical models, which closely replicated the computational designs and the wettability of native airways. Unlike previous bench-top models, our approach offers the first 3D, multi-generation airway tree model with a seamless network of airways and semi-transparent walls to allow convenient imaging of interior of airways.

Our experimental study demonstrated the utility of the models to quantitatively investigate distribution of instilled surfactant in the lungs. We used plug splitting at bifurcations to quantify the effect of orientation of the airway tree on surfactant distribution between the left and right lungs, and within airways of each lung. The split ratios at the $z=0-1$ bifurcation under different orientations were consistent with our previous study using single bifurcating airway models of $z=0-1$ and validated the new model.²³ The airway tree model was particularly useful to study plug splitting at bifurcations of subsequent generations that could not be done with single bifurcating airways. For example, our results showed that orienting the airway tree at $\alpha = 30^\circ$ with respect to the $z=0-1$ plane will cause the instilled surfactant to primarily flow into the left lung. From a practical standpoint, positioning an infant at this orientation may be used to deliver the surfactant solution to the gas exchange units of the left lung and subsequently, the process may be repeated at $\alpha = -30^\circ$ to transport surfactant into the right lung. Thus, targeting both lungs using more than a single instillation may produce a greater therapeutic effect. In addition, consistent with our previous study using single bifurcating airways, orienting the airway tree using a pitch angle of $\phi=15^\circ$ at a constant roll angle of $\alpha=30^\circ$ had a small effect on the split ratio at $z=0-1$ bifurcation. But it generated a significant effect on the distribution of surfactant between the far-left and far-right airways of the left lung. This indicates that a combination of roll and pitch angles can be used to direct the therapeutic solution primarily into a specific portion of the left or right lungs. Such quantitative studies are only feasible with the 3D airway tree model.

In this study, we used airway dimensions of neonates' lungs to establish principles of design of the airway tree and demonstrate the utility of the model for SRT. Nevertheless, our systematic design principles conveniently allow creating airway tree models of different sizes, from babies to adults. These models will be useful for quantitative studies of delivery of fluids such as surfactant solutions and aerosolized therapeutics in the lungs.

Our airway tree model is open to the atmospheric pressure at its end generation, whereas native airways terminate in alveoli. However, alveolar pressure in healthy lungs only changes by ~ 2 cmH₂O with the respect to atmospheric pressure during breathing, i.e., $\sim 0.2\%$ of atmospheric pressure from inspiration to expiration.¹ Thus, our model will provide a reasonably accurate estimate to study plug dynamics in the conducting zone airways ($z=0-7$). In addition, although our model has a significantly larger Young's modulus than native

conducting airways, it mimics the non-compliant conducting airways that do not deform during breathing or SRT due to surrounding cartilage that forms during fetal development.^{1, 37} We also ensured this through image analysis of diameter of airways of excised rat lungs under SRT that did not show a detectable change during ventilation.^{12, 38} Therefore, the difference between stiffness of our physical model and native conducting zone airways is not expected to have a significant effect on fluid motion through the conducting zone airways. However, considering that respiratory zone airways are compliant and deform during breathing, it would be interesting to develop a complete tracheobronchial tree model and investigate whether the compliance of respiratory zone airways and alveoli affects plug dynamics in conducting zone airways under cyclical flow or ventilation. In addition, to mimic ventilation assisted distribution of the instilled surfactant, including pressure sensors in our setup would allow quantitatively study effect of air pressure on surfactant delivery in the lungs. Furthermore, to better mimic geometry of native airways, future studies should consider asymmetry of daughter airways in a generation in terms of length and diameter and changes in the bifurcation angle of airways in the lungs, and investigate the effects on surfactant plug splitting at bifurcations and distribution in the airway tree. To make this approach relevant for personalized medicine, the geometry of airways may be inferred from medical images to develop computational and physical airway tree models tailored to individual patients. Other potential studies that this approach allows are cellularizing airway tree models to study the role of a biologically active layer on the distribution of instilled or inhaled therapeutics, and creating biofilms on airway walls to mimic bacterial insult in the lungs and examining the efficacy of various therapeutics and delivery methods.

Conclusions

We introduced a novel approach to develop a 3D model of human tracheobronchial tree. Using a set of geometric principles obtained from morphometric data of human lungs, we computationally designed a 3D, eight-generation airway tree model and employed additive manufacturing to accurately generate the corresponding physical model. We then employed the physical model to demonstrate the potential for quantitative studies of surfactant delivery in the lungs. Our study showed that the orientation of the airway tree in the gravitational field plays a major role in the distribution of instilled surfactant between the left and right lungs and within each lung. We identified specific orientations that primarily favored surfactant delivery into the left lung or the right lung, and even to a specific lobe of a lung. Our integrated bioengineering approach offers a major tool for quantitative studies of delivery of therapeutic fluids into the lungs to identify conditions that lead to an optimal distribution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support was provided by a University of Akron Firestone Fellowship to H.T.

References

1. West JB Respiratory Physiology: The Essentials. 2015.
2. Andreeva AV, Kutuzov MA, and Voyno-Yasenetskaya TA. Regulation of surfactant secretion in alveolar type II cells. *Am. J. Physiol. Lung Cell Mol. Physiol* 293:L259–71, 2007. [PubMed: 17496061]
3. Veldhuizen EJ, and Haagsman HP. Role of pulmonary surfactant components in surface film formation and dynamics. *Biochim. Biophys. Acta* 1467:255–70, 2000. [PubMed: 11030586]
4. Saad SM, Policova Z, Acosta EJ, and Neumann AW. Effect of surfactant concentration, compression ratio and compression rate on the surface activity and dynamic properties of a lung surfactant. *Biochim. Biophys. Acta* 1818:103–16, 2012. [PubMed: 22020010]
5. Gaver DP 3rd, Samsel RW, and Solway J. Effects of surface tension and viscosity on airway reopening. *J. Appl. Physiol* 69:74–85, 1990. [PubMed: 2394665]
6. Grothberg JB Respiratory fluid mechanics and transport processes. *Annu. Rev. Biomed. Eng* 3:421–57, 2001. [PubMed: 11447070]
7. Tavana H, Huh D, Grothberg JB, and Takayama S. Microfluidics, lung surfactant, and respiratory disorders. *Lab. Med* 40:203–209, 2009.
8. Lewis JF, and Veldhuizen RA. The future of surfactant therapy during ALI/ARDS. *Semin. Respir. Crit. Care Med* 27:377–88, 2006. [PubMed: 16909371]
9. Stevens TP, and Sinkin RA. Surfactant replacement therapy. *Chest*. 131:1577–82, 2007. [PubMed: 17494810]
10. Lista G, Castoldi F, Bianchi S, and Cavigioli F. Surfactant and mechanical ventilation. *Acta Biomed.* 83 Suppl 1:21–3, 2012.
11. Polin RA, and Carlo WA. Surfactant replacement therapy for preterm and term neonates with respiratory distress. *Pediatrics*. 133:156–63, 2014. [PubMed: 24379227]
12. Cassidy KJ, Bull JL, Glucksberg MR, Dawson CA, Haworth ST, Hirschl R, Gavriely N, and Grothberg JB. A rat lung model of instilled liquid transport in the pulmonary airways. *J. Appl. Physiol* 90:1955–67, 2001.
13. Borgas MS, and Grothberg JB. Monolayer flow on a thin film. *J. Fluid Mech* 193:151–170, 1988.
14. Gaver DP, and Grothberg JB. Droplet spreading on a thin viscous film. *J. Fluid Mech* 235:399–414, 1992.
15. Cassidy KJ, Gavriely N, and Grothberg JB. Liquid plug flow in straight and bifurcating tubes. *J. Biomech. Eng* 123:580–9, 2001. [PubMed: 11783729]
16. Halpern D, Jensen OE, and Grothberg JB. A theoretical study of surfactant and liquid delivery into the lung. *J. Appl. Physiol* 85:333–352, 1998. [PubMed: 9655794]
17. Zheng Y, Fujioka H, Grothberg JC, and Grothberg JB. Effects of inertia and gravity on liquid plug splitting at a bifurcation. *J. Biomech. Eng* 128:707–716, 2006. [PubMed: 16995757]
18. Filoche M, Tai CF, and Grothberg JB. Three-dimensional model of surfactant replacement therapy. *Proc. Natl. Acad. Sci. U. S. A* 112:9287–92, 2015. [PubMed: 26170310]
19. Fujioka H, and Grothberg JB. Steady propagation of a liquid plug in a two-dimensional channel. *J. Biomech. Eng* 126:567–77, 2004. [PubMed: 15648809]
20. Fujioka H, and Grothberg JB. The steady propagation of a surfactant-laden liquid plug in a two-dimensional channel. *Phys. Fluids* 17, 2005.
21. Ghadiali SN, and Gaver DP 3rd. An investigation of pulmonary surfactant physicochemical behavior under airway reopening conditions. *J. Appl. Physiol* 88:493–506, 2000. [PubMed: 10658016]
22. Perun ML, and Gaver DP 3rd. An experimental model investigation of the opening of a collapsed untethered pulmonary airway. *J. Biomech. Eng* 117:245–53, 1995. [PubMed: 8618375]
23. Copploe A, Vatani M, Amini R, Choi JW, and Tavana H. Engineered airway models to study liquid plug splitting at bifurcations: Effects of orientation and airway size. *J. Biomech. Eng* 140, 2018.
24. Baroud CN, Tsikata S, and Heil M. The propagation of low-viscosity fingers into fluid-filled branching networks. *J. Fluid Mech* 546:285–294, 2006.

25. Tavana H, Kuo CH, Lee QY, Mosadegh B, Huh D, Christensen PJ, Grotberg JB, and Takayama S. Dynamics of liquid plugs of buffer and surfactant solutions in a micro-engineered pulmonary airway model. *Langmuir*. 26:3744–52, 2010. [PubMed: 20017471]
26. Atefi E, Mann JA Jr., and Tavana H. Ultralow interfacial tensions of aqueous two-phase systems measured using drop shape. *Langmuir*. 30:9691–9, 2014. [PubMed: 25068649]
27. Weibel ER, and Gomez DM. Architecture of the human lung. Use of quantitative methods establishes fundamental relations between size and number of lung structures. *Science*. 137:577–85, 1962. [PubMed: 14005590]
28. Phalen RF, Yeh HC, Schum GM, and Raabe OG. Application of an idealized model to morphometry of the mammalian tracheobronchial tree. *Anat. Rec* 190:167–76, 1978. [PubMed: 629400]
29. Sauret V, Halson PM, Brown IW, Fleming JS, and Bailey AG. Study of the three-dimensional geometry of the central conducting airways in man using computed tomographic (CT) images. *J. Anat* 200:123–34, 2002. [PubMed: 11895110]
30. Kitaoka H, Takaki R, and Suki B. A three-dimensional model of the human airway tree. *J. Appl. Physiol* 87:2207–17, 1999. [PubMed: 10601169]
31. Choi JW, Kim H-C, and Wicker R. Multi-material stereolithography. *J. Mater. Proc. Technol* 211:318–328, 2011.
32. Hope RL, Roth RN, and Jacobs PA. Adaptive slicing with sloping layer surfaces. *Rapid Prototyping J* 3:89–98, 1997.
33. Sabourin E, Houser SA, and Bøhn JH. Adaptive slicing using stepwise uniform refinement. *Rapid Prototyping J*. 2:20–26, 1996.
34. Lasalvia M, Castellani S, D'Antonio P, Perna G, Carbone A, Colia AL, Maffione AB, Capozzi V, and Conese M. Human airway epithelial cells investigated by atomic force microscopy: A hint to cystic fibrosis epithelial pathology. *Exp. Cell Res* 348:46–55, 2016. [PubMed: 27590528]
35. Petrak D, Atefi E, Yin L, Chilian W, and Tavana H. Automated, spatio-temporally controlled cell microprinting with polymeric aqueous biphasic system. *Biotechnol. Bioeng* 111:404–12, 2014. [PubMed: 23983007]
36. Grotberg JB Respiratory fluid mechanics. *Phys. Fluids* 23:21301, 2011.
37. Szpinda M, Daroszewski M, Wozniak A, Szpinda A, and Mila-Kierzenkowska C. Tracheal dimensions in human fetuses: an anatomical, digital and statistical study. *Surg. Radiol. Anat* 34:317–323, 2012. [PubMed: 21984196]
38. Anderson JC, Molthen RC, Dawson CA, Haworth ST, Bull JL, Glucksberg MR, and Grotberg JB. Effect of ventilation rate on instilled surfactant distribution in the pulmonary airways of rats. *J. Appl. Physiol* 97:45–56, 2004. [PubMed: 14990558]

Figure 1.

(a) Measurements of lengths of daughter plugs (a) after the parent plug splits at the $z=0-1$ generation, (b) at the $z=1-2$ generation of the left lung, and (c) at the $z=1-2$ generation of the right lung.

Figure 2.

(a) Computational model of a single tapering tube, (b-g) step-wise process of creating a single bifurcating airway unit from a single tube.

Figure 3.

Process of computational design of parts that are used to create a multi-generation airway tree, (a-e) A single airway from each generation is added and starting from generation $z=3$, airways also rotate 90° . (f-i) Starting from the generation $z=0$, airway tubes are removed one generation at a time and the remaining structure at each step is saved as a part for later use in the assembly process. Only three steps of removal ($z=0$, $z=1$, and $z=2$) are shown.

Figure 4. Assembly process to create a computational model of lung airway tree. (a-c) The first three steps of connecting parts. (d) The resulting symmetric airway tree model contains 255 airway tubes representing generations $z=0-7$.

Figure 5.
Models of airways fabricated using additive manufacturing of computational designs.

Figure 6.

(a) An Infasurf plug formed in the tracheal tube, (b) the plug after splitting at the $z=0-1$ bifurcation at a horizontal orientation ($\alpha=\varphi=0^\circ$), and (c) the plug after splitting at the $z=1-2$ bifurcation at $\alpha=30^\circ$ and $\varphi=0^\circ$. (d-e) Splitting of an Infasurf plug in the $z=1-2$ bifurcation of (d) the left lung and (e) the right lung at $\alpha=30^\circ$ and $\varphi=0^\circ$.

Figure 7.

Split ratios under different roll angles and zero pitch angle are shown versus Capillary number for z=0–1 bifurcation, z=1–2 bifurcation of the left lung, and z=1–2 bifurcation of the right lung.

Figure 8.

Effect of orienting airways through a pitch angle of $\varphi=15^\circ$ is shown on the split ratios for z=0–1 bifurcation, z=1–2 bifurcation of the left lung, and z=1–2 bifurcation of the right lung.